RESEARCH NOTE

Emergence of a serotype 1 *Streptococcus pneumoniae* lineage colonising healthy children in Portugal in the seven-valent conjugate vaccination era

S. Nunes[1], *R. Sá-Leão*[1], *L. C. Pereira*[1] *and H. de Lencastre*[1,2]

[1]Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal,
[2]Laboratory of Microbiology, The Rockefeller University, New York, USA

ABSTRACT

Serotype 1 pneumococci are rarely isolated from carriers, but are an important cause of pneumococcal invasive disease in many regions of the world. This report describes the emergence and expansion of a single serotype 1 lineage (characterised by multilocus sequence type 306) among healthy carriers attending day-care centres in Portugal. The prevalence of serotype 1 strains among all pneumococci increased from 0% in 2001 and 2002, to 0.4% in 2003, and 3.1% in 2006. These observations paralleled the introduction and increased use of the seven-valent pneumococcal conjugate vaccine in the study group, suggesting a direct relationship between these events.

Keywords Carriage, colonisation, conjugate vaccine, pneumococci, serotype 1, vaccine

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Infections caused by *Streptococcus pneumoniae* result in significant morbidity and mortality in children [1]. More than 90 pneumococcal serotypes have been identified, based on the structure of the capsular polysaccharide [2], and the potential of pneumococcal isolates to cause invasive disease has been associated with the capsule expressed [3]. Serotype 1 is considered to be a primary pathogen, as it is isolated consistently from cases of invasive disease and is rarely found among asymptomatic carriers, even in geographical areas where it is a dominant cause of pneumococcal invasive disease [4–7]. Since colonisation precedes disease, it has been proposed that rarely carried serotypes, e.g., serotype 1, are poor colonisers because of a short duration of colonisation and/or a low density [8].

In the first half of the 20th century, serotype 1 was associated with outbreaks of invasive disease among adults in the western world [9]. Recent reports from Africa have described an ongoing highly lethal epidemic of pneumococcal meningitis caused by serotype 1 isolates of the ST217 clonal complex, as determined by multilocus sequence typing [10,11]. In Portugal, the rest of Europe and North America, serotype 1 pneumococcal disease has been associated with ST306 and its related sequence types [12,13]. The present study describes the emergence and clonal structure of serotype 1 isolates among Portuguese asymptomatic carriers, and considers the events that may have led to its emergence.

Nasopharyngeal samples were obtained from children attending day-care centres in Lisbon and Oeiras, Portugal, between January and March in 2001, 2002, 2003 and 2006 (Table 1). The ages of the children ranged from 4 months to 6 years. Pneumococci were isolated and identified on the basis of selective growth on gentamicin blood agar plates, optochin susceptibility, colony morphology, and α-haemolysis [14]. Capsular typing was performed by multiplex PCR [15] or, for those isolates for which the serotype could not be determined by PCR, the Quellung reaction using specific antisera (Statens Serum Institute, Copenhagen, Denmark). Isolates were tested for susceptibility to erythromycin, clindamycin, tetracycline, chloramphenicol, co-trimoxazole and levofloxacin by disk-diffusion following CLSI guidelines [16], and for penicillin and ceftriaxone by Etest (AB Biodisk, Solna, Sweden). Pulsed-field gel electrophoresis (PFGE) was performed for serotype 1 strains as described previously [17], and a dendrogram was generated using Bionumerics software (Applied Maths, Sint-Martins-Latem, Belgium) [18]. Multilocus sequence typing was performed as described previously [14] for repre-

Corresponding author and reprint requests: R. Sá-Leão, Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica, Rua da Quinta Grande, Oeiras, 2780-156 Oeiras, Portugal
E-mail: rsaleao@itqb.unl.pt

Table 1. Study population and distribution of serotype 1 isolates

Year	Children sampled	No. of pneumococcal isolates	No. of serotype 1 isolates (%)[a]	Day-care centres with serotype 1 isolates
2001	717	465	0	0/11
2002	834	559	0	0/14
2003	766	557	2 (0.4)	2/14
2006	571	392	12 (3.1)	4/12

[a]Percentage of serotype 1 isolates among all pneumococci.

sentative isolates of the different PFGE subtypes, choosing one isolate per collection year. Serotypes and molecular types of antimicrobial-resistant pneumococci recovered in 2001, 2002 and 2003 (which did not include any serotype 1 strains) have been described previously [18,19].

Of 1551 nasopharyngeal samples obtained from children in 2001 and 2002, 1075 yielded pneumococci, none of which was of serotype 1 (Table 1). In 2003, two serotype 1 isolates (representing 0.4% of all pneumococci) were recovered from two children attending different day-care centres. In 2006, 12 isolates (3.1% of all pneumococci) were found to be of serotype 1, and these were isolated from 12 children attending four day-care centres (Table 1). The 14 serotype 1 isolates were fully susceptible to all antimicrobial agents tested. According to PFGE, they belonged to a single cluster with two PFGE subtypes (Fig. 1). According to multilocus sequence typing, this cluster was associated with ST306 and the related ST228 (double-locus variant of ST306), which is found commonly among serotype 1 disease-causing isolates from Portugal and the rest of Europe [8]. All children carrying serotype 1 were aged >2 years, and six had been vaccinated with the seven-valent pneumococcal vaccine (PCV7). Thus, the emergence of serotype 1 among healthy Portuguese children in 2003–2006 was associated with a single pneumococcal lineage that is disseminated in Europe, and the possibility that the hypervirulent pneumococcal clone causing meningitis in Africa might have been imported into Portugal was excluded. These findings contrast with data from Sweden from the 1990s, where the serotype 1 lineage emerged as a frequent cause of invasive disease, but could not be detected among pneumococcal isolates obtained from children attending Swedish day-care centres [7].

The present study revealed cross-transmission within a day-care centre (DCC 22; Fig. 1), but the serotype 1 clone was also recovered from children attending four other day-care centres. The fact that all carriers were aged >2 years mimicked the age distribution found among serotype 1 isolates causing invasive disease [8]. Indeed, for unknown reasons, serotype 1 does not usually cause disease among infants (neonates excluded). Since all serotype 1 isolates were susceptible to all antimicrobial agents, the possibility of selection caused by antimicrobial pressure could also be excluded.

PCV7 became commercially available in Portugal in June 2001, but is not part of the national vaccination plan and is not subsidised by the state [20]. Although there are no official data concerning the use of PCV7 in Portugal, the proportions of children vaccinated with PCV7 in the study were 12% in 2002, 24% in 2003, and 64% in 2006 (personal unpublished data). These observations suggest that the emergence of serotype 1 may have been related to the impact of PCV7 on colonisation, which is known to lead to serotype replacement of vaccine types by non-vaccine types [21,22]. Whether this emergence resulted from an unmasking effect or a true clonal expansion is unclear [23].

In conclusion, the present study revealed the emergence of a serotype 1 lineage of pneumococci among healthy Portuguese carriers. This

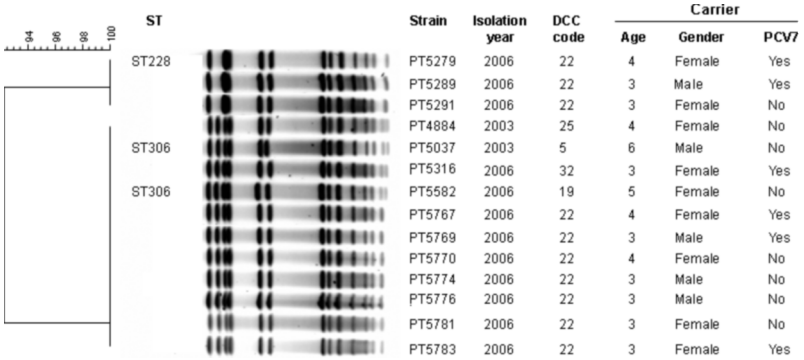

Fig. 1. Genotypic relationship of serotype 1 pneumococcal isolates according to pulsed-field gel electrophoresis. ST, sequence type; DCC, day-care centre; PCV7, vaccinated with seven-valent pneumococcal conjugate vaccine.

emergence occurred soon after the introduction of PCV7 use in Portugal, and the increase in the prevalence of serotype 1 accompanied the increased use of PCV7. Since this serotype has been associated with outbreaks of fatal meningitis and complicated pneumonia, and is not included in PCV7, it will be important to closely survey the expansion and evolution of these strains among healthy carriers, as this group is known to be the major reservoir of pneumococci.

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